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**Scarce population genetic differentiation but substantial spatiotemporal phenotypic variation of water-use efficiency in *Pinus sylvestris* at its western distribution range**

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## Abstract

Water and carbon fluxes in forests are largely related to leaf gas exchange physiology varying across spatiotemporal scales and modulated by plant responses to environmental cues. We quantified the relevance of genetic and phenotypic variation of intrinsic water-use efficiency ( $WUE_i$ , ratio of net photosynthesis to stomatal conductance of water) in *Pinus sylvestris* L. growing in the Iberian Peninsula as inferred from tree-ring carbon isotopes. Inter-population genetic variation, evaluated in a provenance trial comprising Spanish and German populations, was low and relevant only at continental scale. In contrast, phenotypic variation, evaluated in natural stands (at spatial level) and by tree-ring chronologies (at temporal inter-annual level), was important and ten- and three-fold larger than the population genetic variance, respectively. These results points to preponderance of plastic responses dominating variability in  $WUE_i$  for this species. Spatial phenotypic variation in  $WUE_i$  correlated negatively with soil depth ( $r = -0.66$ ;  $p < 0.01$ ), while temporal phenotypic variation was mainly driven by summer precipitation. At the spatial level,  $WUE_i$  could be scaled-up to ecosystem-level WUE derived from remote sensing data by accounting for soil water holding capacity ( $r = 0.63$ ;  $p < 0.01$ ). This outcome demonstrates a direct influence of the variation of leaf-level  $WUE_i$  on ecosystem water and carbon balance differentiation. Our findings highlight the contrasting importance of genetic variation (negligible) and plastic responses in  $WUE_i$  (large, with changes of up to 33% among sites) on determining carbon and water budgets at stand and ecosystem scales in a widespread conifer such as *Pinus sylvestris*.

**Keywords:** genetic variation; phenotypic plasticity; *Pinus sylvestris*; remote sensing; tree rings; intrinsic water-use efficiency

## Introduction

The carbon and water cycles on Earth are coupled with the composition and functioning of forest ecosystems (Pan et al. 2011; Ukkola et al. 2016). Forests represent a net sink of  $1.1 \times 10^{15}$  g of carbon per year (Pan et al. 2011) and contribute to *ca.* 56% of terrestrial evapotranspiration (Schlesinger and Jasechko 2014). Ecosystem water-use efficiency (WUE), defined as the ratio between gross primary productivity and evapotranspiration, is a key characteristic determining the carbon and water balance of forests. Spatial heterogeneity, temporal fluctuations and their interactions over a broad spectrum of scales determine the multifaceted variability of WUE (e.g. Saurer et al. 2014). In order to monitor climate-vegetation feedbacks, an in-depth understanding of factors and processes that determine variation of WUE has become an urgent priority in ecology and biogeosciences (Keenan et al. 2013; Knauer et al. 2016).

Attempts to relate ecosystem WUE to leaf-level plant responses are pursued by the estimation of intrinsic water-use efficiency ( $WUE_i$ ), or the ratio of net photosynthesis to stomatal conductance of water. The diffusion equation [ $WUE_i = A/g_s = (C_a - C_i)/1.6$ ] links carbon assimilation rate ( $A$ ) to stomatal conductance ( $g_s$ ) to the difference in atmospheric ( $C_a$ ) and inner leaf ( $C_i$ )  $CO_2$  concentration, irrespective of atmospheric water demand (Farquhar et al. 1989a). Detailed information on leaf-level physiology can be gained through the analysis of stable isotopes in tree rings (McCarroll and Loader 2004). Particularly, the ratio of the heavy to light carbon isotopes ( $^{13}C/^{12}C$ ) depends on factors affecting  $CO_2$  uptake, being directly related to  $WUE_i$  (Farquhar et al. 1989b). Alternatively, eddy covariance fluxes, remote sensing data and dynamic global vegetation models provide estimates of WUE at ecosystem scale (e.g. Frank et al. 2015; Dekker et al. 2016).

The spatiotemporal dynamics of WUE are conditional to the functional characteristics of the vegetation and, particularly, of the dominant tree species as fundamental component of

74 forests. In this regard, it is expected that  $WUE_i$  fluctuations of a prevailing tree species could  
75 be a determinant factor of WUE variation, hence strongly influencing water and carbon  
76 economy of the whole ecosystem. Quantifying the spatial and temporal dimensions of the  
77 variation of a functional characteristic such as  $WUE_i$  in forest trees is experimentally  
78 challenging. Trees can respond to changes in the environment through phenotypic plasticity,  
79 i.e. the array of phenotypes that an individual shows in response to different growing  
80 conditions (Nicotra et al. 2010). Phenotypic plasticity allows plants to adjust their functional  
81 traits to environmental changes, up to a level in which the extent of plastic responses (or  
82 range of potential acclimation) does not suffice to cope with substantial variations in external  
83 factors (Bussotti et al. 2015). Concurrently, different selective pressures may also engender  
84 variation in functional traits within the genetic pool of a species through localised selection of  
85 genotypes that perform better under particular conditions (i.e. genetic adaptation, Alberto et  
86 al. 2013). Intra-specific phenotypic variation of a functional trait is thus the result of the co-  
87 occurring effects of phenotypic plasticity and genetic adaptation, which have been long  
88 proposed as partially independent mechanisms shaping plants' responses to the environment  
89 (Bradshaw, 1965).

90         So far, a comprehensive investigation of the magnitude of phenotypic variation and its  
91 components (spatiotemporal and genetic) is mostly lacking for  $WUE_i$ , even for widely  
92 distributed tree species. The analysis of genotype by environment interaction in multi-  
93 environment trials (i.e. the assessment of the performance of individuals in common gardens  
94 across different environments) is well suited to this task, but their availability in forest trees is  
95 limited and their records are often extremely unbalanced, which further complicates  
96 partitioning such effects in a straightforward manner.

97         In this study, we focus on Scots pine (*Pinus sylvestris* L.), the most widespread conifer  
98 of the Northern Hemisphere. Scots pine is found under very diverse climatic conditions and

ecological habitats from Boreal forests at high latitudes to Alpine biomes and meso-Mediterranean areas at mid-latitudes, with some populations at the trailing edge of distribution (e.g., Mediterranean basin) subjected to chronic summer drought (Irvine et al. 1998; Sánchez-Salguero et al. 2015). As most pines, Scots pine is considered a drought-avoidant and isohydric species (Irvine et al. 1998), that is, prone to close stomata under water shortage to maintain approximately constant leaf water potentials. During the last decades, the southernmost populations of this species have experienced drought-induced decline in growth and die-back episodes (Hereş et al. 2012; Camarero et al. 2018). Data derived from common garden experiments indicate the existence of inter-population genetic differentiation in traits such as nutrient acquisition (Oleksyn et al. 2003), phenology (Notivol et al. 2007), survival (Benito-Garzón et al. 2011) or carbon allocation (Bachofen et al. 2018). Some evidences also suggest specific adaptation of southern populations to drought (Taeger et al. 2013; Matías et al. 2014). In addition to genetic differentiation, phenotypic plasticity drives variation in traits such as hydraulic conductivity (Irvine et al. 1998), radial growth and carbon isotope composition (Eilmann et al. 2010), and gas exchange physiology (Feichtinger et al. 2017) in this species.

Despite the wealth of information on functional traits for *P. sylvestris*, little is known about how phenotypic variation of  $WUE_i$  is structured for this species. A few studies based on carbon isotopes have investigated the relevance of genetic variation of  $WUE_i$  through common garden tests and clone bank experiments (Palmroth et al. 1999; Brendel et al. 2002), finding low intra-specific genetic variation for this trait. However, these studies did not include populations from the trailing edge of distribution of the species, where trees are subjected to frequent summer droughts (Sánchez-Salguero et al. 2015). A persistent selective pressure on water conservation might have led to an appreciable population genetic differentiation in  $WUE_i$ . Conversely, Brendel et al. (2002) concluded that the main source of

phenotypic variation of  $WUE_i$  is related to a plastic response of individuals to climate, as expected for a species with high stomatal sensitivity to water shortage (Irvine et al. 1998), which can be found across a vast array of conditions and environments. However, the magnitude and relevance of the spatial and temporal components of phenotypic variation remain unclear. Several studies have characterized temporal variation of  $WUE_i$  in *P. sylvestris* (e.g. Andreu-Hayles et al. 2011; Voltas et al. 2013), but mainly focusing on the long-term consequences of rising atmospheric  $CO_2$  and climate on tree performance rather than on interpreting the relevance of temporal variation in relation to spatial and genetic effects.

Here, we use carbon isotope discrimination ( $\Delta^{13}C$ ) in tree rings sampled in a provenance trial and in natural stands of *P. sylvestris* aiming at quantifying the importance of inter-population genetic variation of  $WUE_i$  in relation to the overall spatiotemporal phenotypic variation of the species near its south-western distribution limit (Pyrenees range, north-eastern Iberian Peninsula). Our main objective is to understand how phenotypic variation in carbon assimilation rate and stomatal conductance is structured in a widespread conifer and to identify the external drivers of such changes. We hypothesize that: 1) the inter-population genetic variation of  $WUE_i$  is of little relevance for *P. sylvestris* owing to a reduced selective pressure towards water conservation as compared with other pine species that are more xeric and, therefore, subjected to extreme and recurrent drought events (e.g. *Pinus halepensis* [Voltas et al. 2008]); and 2) the spatiotemporal phenotypic variation of  $WUE_i$  is of comparatively much more relevance than the extent of population genetic differentiation, mainly owing to the relevance of plastic effects for this functional trait. To better understand how leaf-level responses of a dominant tree species may contribute to the overall water and carbon balance in conifer forests,  $WUE_i$  records of phenotypic variation obtained across natural stands are compared to several indicators of ecosystem-level WUE derived from



satellite data. In this way, we evaluate the feasibility of upscaling tree-ring-based high-resolution spatial estimates of  $WUE_i$  over the whole forest stand.

## Material and methods

### Provenance trial

A provenance trial located in Aragiúes del Puerto (NE Iberian Peninsula; 42°44'N, 00°37'W, 1350 m a.s.l.) and comprising sixteen Spanish and six German populations (Fig. 1a; Online Resource 1) was sampled to quantify the importance of inter-population genetic differentiation of  $WUE_i$ . The mean annual temperature at the site is 7.4°C and the mean annual precipitation is 1086 mm (Fig. 1a), of which *ca.* 22% fall in summer (1960-1990 period) (WorldClim database; Hijmans et al. 2005). The trial has optimal growing conditions for this species in the Iberian Peninsula, and can be considered representative of the average (albeit slightly warmer) climate conditions encountered by *P. sylvestris* across Europe (Fig. 1b). Two-years-old seedlings were planted in 1992 according to a randomized complete block design with four replicates. Each replicate plot consisted of 16 trees of the same population, spaced  $2.5 \times 2.5$  m, resulting in square-shaped experimental units.

The Spanish populations are representative of the species distribution in the Iberian Peninsula (south-western edge of its present range). The German populations are distributed across the country (i.e. approximately the centre of the European distribution range for this species) and were chosen to compare their performance against those of the Spanish populations (for further details see Alía et al. [2001]) (Fig. 1b). At age 20 (year 2010), survival rate (%) per replicate plot was recorded and height and diameter at breast height (DBH) were measured using telescopic measuring sticks and tapes respectively. Mean tree-ring width (TRW) of each tree was estimated as the radius divided by age. At age 25 (year 2015), wood cores were sampled at 1.3 m using 5-mm Pressler increment borers. We selected two adjacent blocks having relatively uniform growing conditions and six trees per population

174 were sampled (i.e. three trees per block). Tree rings were visually cross-dated and rings  
175 corresponding to the 2005-2014 period were pooled together and used for carbon isotope  
176 analysis.

## 178 **Natural stands**

179 The spatial extent of phenotypic variation in  $WUE_i$ , which comprises genetic variation  
180 and phenotypic plasticity, was evaluated in natural stands of *P. sylvestris*. The sampling sites  
181 consisted of 30 unmanaged, monospecific and even-aged stands located in the central and  
182 eastern Pyrenees mountains (north-eastern Iberian Peninsula) (Fig. 1a; Online Resource 2).  
183 The stands cover a wide range of local climatic, physiographic and edaphic conditions (Fig.  
184 1b; Online Resource 2). They are supposed to share adaptive characteristics as they belong to  
185 two neighbouring Spanish provenance regions (or adaptive units in which phenotypically or  
186 genetically similar stands are found; European Council Directive 1999/105/EC). Each stand  
187 was characterized for the following features: elevation, slope, hillslope position (footslope,  
188 backslope or shoulder) and aspect, carbonate content in soil, stand density, stand basal area  
189 and soil depth (maximum dig depth before finding a compact rock layer). The Harmonized  
190 World Soil Database (FAO, 2009) classifies the soils of the stands as humic or calcareic  
191 cambisols with a resolution of 1 km<sup>2</sup>. Soil texture is relatively uniform among sites (range =  
192 37-42% sand, 23-44% silt and 19-36% clay).

193 Wood cores were sampled at 1.3 m using 5-mm Pressler increment borers in 2008. A  
194 variable number of representative trees (two to ten) were taken per stand (tree age =  $68 \pm 18$   
195 years; mean  $\pm$  SD). Tree rings were visually cross-dated and tree age was calculated. The  
196 rings corresponding to the period of 2000-2007 were identified and used to estimate mean  
197 tree-ring width (TRW). These rings were then pooled together and used for carbon isotope  
198 analysis. Height and DBH of sampled trees were also measured.

## Tree-ring chronologies

At temporal level, the magnitude of phenotypic variation was evaluated in two representative sites (Arcalís, 42°22'N, 01°11'E, 1150 m a.s.l.; Seira, 42°31'N, 0°23'E, 1538 m a.s.l.) out of the 30 sites comprising the sampled region (Fig. 1a; Online Resource 2). Tree-ring chronologies were built in these sites for carbon isotopes analysis. Arcalís is drier and warmer compared with Seira (Fig. 1b) and is located in an area where recent die-off episodes have been observed (Hereş et al. 2012). Wood cores were sampled from 40 and 20 representative, adult and healthy trees in Arcalís and Seira respectively. Tree rings were cross-dated and TRW was measured using WinDENDRO™ (Regent Instruments Inc., Ville de Québec, Canada) with a resolution of 0.01 mm. The accuracy of cross-dating was checked with the COFECHA program (Holmes 1983). The quality of the resulting chronologies was evaluated by the expressed population signal (EPS) statistic, which resulted adequate (>0.85) for the study period (1975-2009). Individual rings from a subset of ten cores in Arcalís and five cores in Seira were separated with a scalpel under a binocular microscope to carry out carbon isotope analysis with annual resolution. Approximately the last 30-35 rings (corresponding to 1975-2008 for Arcalís and 1980-2009 for Seira) were used for isotopic analysis, hence avoiding juvenile imprints on the carbon isotope signature (tree age =  $68 \pm 19$  and  $66 \pm 11$  years in Arcalís and Seira respectively; mean  $\pm$  SD).

## Carbon isotope analysis and leaf-level intrinsic water-use efficiency

Wood samples (i.e. pooled rings from the provenance trial and sampled sites, annual rings from tree-ring chronologies) were milled to a fine powder with a mixer mill (Retsch MM301, Haan, Germany) and purified to  $\alpha$ -cellulose (Ferrio and Voltas 2005). An aliquot of 0.9-1.1 mg of  $\alpha$ -cellulose was weighted and encapsulated into tin capsules that underwent combustion using a Flash EA-1112 elemental analyser interfaced with a Finnigan MAT Delta

C isotope ratio mass spectrometer (Thermo Fisher Scientific Inc., MA, USA). Carbon isotope composition ( $\delta^{13}\text{C}$ ) was calculated as:

$$\delta^{13}\text{C} (\text{‰}) = (R_{\text{sample}} / R_{\text{standard}} - 1) \times 1000 \quad (1)$$

where  $R_{\text{sample}}$  and  $R_{\text{standard}}$  are the isotope ratios ( $^{13}\text{C}/^{12}\text{C}$ ) of the sample and of the Vienna Pee Dee Belemnite (VPDB) standard respectively.

The  $\delta^{13}\text{C}$  values were then used to estimate carbon isotope discrimination ( $\Delta^{13}\text{C}$ ) following Farquhar et al. (1989b):

$$\Delta^{13}\text{C} (\text{‰}) = (\delta^{13}\text{C}_a - \delta^{13}\text{C}_s) / (1 + \delta^{13}\text{C}_s / 1000) \quad (2)$$

where  $\delta^{13}\text{C}_a$  and  $\delta^{13}\text{C}_s$  are the carbon isotope composition of atmospheric  $\text{CO}_2$  and sample respectively.  $\delta^{13}\text{C}_a$  was inferred by interpolating a range of data from Antarctic ice-core records, as described by Ferrio et al. (2005). According to these records, the  $\delta^{13}\text{C}_a$  value applied to  $\alpha$ -cellulose  $\delta^{13}\text{C}$  was -8.20‰ (provenance trial samples; 2005-2014 period), -8.09‰ (sampled sites; 2000-2007 period) and ranged between -7.30‰ and -8.23‰ (annually-resolved tree rings; 1975-2009 period).

$\Delta^{13}\text{C}$  is proportional to the ratio of intercellular ( $C_i$ ) to atmospheric ( $C_a$ )  $\text{CO}_2$  concentrations in C3 plants as follows:

$$\Delta^{13}\text{C} (\text{‰}) \approx a + (b - a) \times (C_i / C_a) \quad (3)$$

where  $a$  is the fractionation during diffusion through stomata ( $\sim 4.4\text{‰}$ ) and  $b$  is the fractionation due to carboxylation by Rubisco ( $\sim 27\text{‰}$ ) (Farquhar et al. 1989b). The previous equation is valid for the primary photosynthetic assimilates, but it does not consider further fractionation occurring downstream from photosynthesis to  $\alpha$ -cellulose formation (Gessler et al. 2014). Indeed, leaves are usually less enriched in  $^{13}\text{C}$  than wood  $\alpha$ -cellulose. To estimate  $\Delta^{13}\text{C}$  at the leaf level, we added a value of +2.1‰ to our  $\alpha$ -cellulose  $\Delta^{13}\text{C}$  values, as average difference between leaf  $\Delta^{13}\text{C}$  and  $\alpha$ -cellulose  $\Delta^{13}\text{C}$  reported by Frank et al. (2015). We assumed near-constancy of differences between chloroplastic and intercellular  $\text{CO}_2$

concentration at the intra-specific level and a linear relationship between  $g_s$  and internal conductance in response to varying water availability. Anyhow, the high internal conductance of *Pinus sylvestris* suggests low mesophyll limitations of photosynthesis (Vernomann-Jürgenson et al. 2017).

WUE<sub>i</sub> was obtained from leaf-level  $\Delta^{13}\text{C}$  according to Farquhar et al. (1989b):

$$\text{WUE}_i = (C_a \times (b - \Delta^{13}\text{C})) / (1.6 \times (b - a)) \quad (4)$$

$C_a$  was obtained from National Oceanic and Atmospheric Administration (NOAA) Earth System Research Laboratory data (<http://www.esrl.noaa.gov/>). We considered the average  $C_a$  corresponding to the time period of the analysed tree rings (ten years for the provenance trial, eight years for the sampled sites and annually-resolved values for characterising temporal variation). Further analyses were performed using leaf-level WUE<sub>i</sub> estimates.

## Climate data

Long-term (1960-1990) averages of precipitation, maximum and minimum temperatures were obtained on a monthly basis from the WorldClim database with a resolution of 1 km<sup>2</sup> (Hijmans et al. 2005). They were used to infer mean annual temperature ( $T_{an}$ ), total annual precipitation ( $P_{an}$ ) and summer (June to August) precipitation ( $P_s$ ) of each geographic origin of populations evaluated at the provenance trial (Online Resource 1) and of each natural stand (Online Resource 2). Annual potential evapotranspiration ( $PET_{an}$ ) was derived from monthly temperatures and precipitation according to the Hargreaves method (Hargreaves and Samani 1982). Climate at the Spanish provenances is in general less humid than that of German provenances. Particularly, Spanish populations originated from climates having a significantly lower  $P_s$  ( $143 \pm 49$  mm vs.  $242 \pm 89$  mm respectively; mean  $\pm$  SD), lower  $P_s$  to  $P_{an}$  ratio ( $0.19 \pm 0.16$  vs.  $0.32 \pm 0.21$ ) and higher  $PET_{an}$  ( $880 \pm 69$  mm vs.  $762 \pm 62$  mm) than

their German counterparts ( $p < 0.05$ ; two-tailed  $t$ -tests). In comparison, the climate conditions in the provenance trial are intermediate between those of German and Spanish populations ( $P_s = 238$  mm,  $P_s$  to  $P_{an}$  ratio = 0.22,  $PET_{an} = 747$  mm).

Precipitation, temperature and the Standardised Precipitation-Evapotranspiration Index (SPEI) corresponding to the site chronologies were obtained on a monthly basis from the CRU TS3.22 dataset (Harris et al. 2014). CRU TS3.22, despite its lower spatial resolution compared with WorldClim, provides yearly records of monthly climate data suitable to investigate relationships between climate and annually-resolved  $WUE_i$  estimates. The climate records, available on a  $0.5^\circ$  latitude/longitude grid for global land areas, covered the period in which annual estimates of  $WUE_i$  were available (1975-2008 for Arcalís, 1980-2009 for Seira).

## Statistical analyses

The magnitudes of population genetic differentiation and phenotypic variation (spatial and temporal) in  $WUE_i$  were quantified independently using the three different sources of data: provenance trial for population differentiation, natural stands for spatial variation, and tree-ring chronologies for temporal variation. To this end, we used linear mixed-effects or random-effects models through restricted maximum likelihood (REML) (see below for a detailed description). The significance of variance components of random effects was evaluated through likelihood ratio tests. These models were fitted using SAS/STAT (ver. 9.4, SAS Inc., Cary, NC, USA). The suitability of analysis of variance (ANOVA) was evaluated through normal quantile plots of the residuals (Online Resource 3).

### *Provenance trial*

The provenance trial was subjected to mixed-effects analysis of variance (ANOVA) with a fixed block effect, a random population effect, a random block  $\times$  population interaction

(intra-block error) and a random variation of trees within a plot (intra-plot error or residual). The random population term provided an estimate for the variance component associated to inter-population genetic variation in  $WUE_i$ . This term was further partitioned into a fixed term accounting for differences between large regions (or countries of origin, Germany vs. Spain) and a random effect quantifying the variation among populations nested to country of origin. This was done to determine the extent by which population genetic differentiation for the trait could be attributed to differences between contrasting ecoregions. An equivalent procedure was also applied to tree height, mean TRW and survival (expressed in per cent values on a plot basis). In this last case, an angular transformation was used to achieve normality.

### *Natural stands*

A full random-effects ANOVA was fitted to  $WUE_i$  and TRW records testing for significant variation among stands. The stand (site) effect was declared as random and the associated variance component was used as an estimate of the spatial dimension of phenotypic variation for these traits. The random variation of trees within a site was used as residual or error term. We used simple correlations to test for the effects of physiography, climate and soil characteristics on site  $WUE_i$  and TRW. The associations between  $WUE_i$ , TRW and height, and the effect of tree age on  $WUE_i$  and TRW were also tested at tree level by simple correlations.

### *Tree-ring chronologies*

$WUE_i$  and TRW time series of the site chronologies showed long-term trends (Online Resource 3). For the purpose of studying high-frequency variation in  $WUE_i$ , estimates were re-calculated considering an average  $C_a$  value for a 30-year period instead of using annually-resolved  $C_a$  records. In this way, the direct effect of  $CO_2$  fertilization enhancing  $WUE_i$  over

time was excluded from the temporal trend. For the same purpose, TRW was investigated by high-pass filtering using cubic smoothing splines with 50% frequency cut-off at 15 years (ARSTAN program; Cook and Krusic 2005). The resulting  $WUE_i$  time series ( $C_a$ -constant  $WUE_i$ ) and the residuals of TRW splines ( $TRW_{res}$ ) were considered free of increased atmospheric  $CO_2$  concentration and ontogenic effects, thus retaining primarily inter-annual variation. Thus, only the fraction of temporal variation associated to climate effects was presumably quantified by mixed-effects ANOVAs. These models consisted of a fixed site chronology effect and random year, random year by site interaction and random tree-within-site effects. The random year by tree-within-site interaction was used as a residual term. The variance component for the year effect was taken as an estimate of the temporal dimension of phenotypic variation for these traits. Indexed TRW records ( $TRW_i$ ) were obtained by applying an autoregressive model to each  $TRW_{res}$  series to remove the autocorrelation related to the growth of the previous year and later calculating the ratio of observed to predicted values of the cubic splines. Bootstrapped correlations between annual  $C_a$ -constant  $WUE_i$  or  $TRW_i$  and monthly climate factors (precipitation, temperature and SPEI) from the October of the previous year to September of the ring year were calculated using the software DendroClim (Biondi and Waikul 2004). Statistical significance of correlations was estimated by drawing 1000 bootstrapped samples with replacement from the initial data set.

### **Ecosystem water-use efficiency**

We correlated the estimates of leaf-level  $WUE_i$  of the natural stands with four ecosystem-level indicators of water-use efficiency. Ecosystem water-use efficiency (EWUE) is a common indicator calculated as the ratio between ecosystem gross primary productivity (GPP) and its associated evapotranspiration (ET) (Huang et al. 2015). Soil water-use efficiency (SWUE) is a recently proposed indicator calculated as the ratio between GPP and



soil water content (SWC) (He et al. 2017). We took advantage of available high-resolution (1 km<sup>2</sup>) remote sensing data to calculate the two indices for each sampled site. Mean GPP and ET data for the period of 2000-2014 were derived from Moderate Resolution Imaging Spectroradiometer (MODIS) on board NASA's Terra satellite, available at <https://daacmodis.ornl.gov/data.html>. Mean SWC for the period of 2010-2016 was derived from the ESA's Soil Moisture and Ocean Salinity (SMOS) mission data, downscaled at 1 km<sup>2</sup> resolution as described in Merlin et al. (2013). SWC data are available at <http://cp34-bec.cmima.csic.es/data/data-access/>. We also calculated an "inherent" WUE (IWUE) multiplying EWUE by the average vapour pressure deficit (VPD) estimated at each site. IWUE accounts for atmospheric water demand and it approximates WUE<sub>i</sub> better compared with EWUE (Beer et al. 2009). Average VPD was calculated from altitude and monthly temperature and precipitation following Ferrio and Voltas (2005). As SWC is given in relative units as m<sup>3</sup> of water per m<sup>3</sup> of soil, we estimated the total amount of water available along the soil profile by multiplying SWC by soil depth at site level. We then recalculated SWUE as the ratio between GPP and the corrected (total) SWC (SWUE'). Finally, we correlated leaf-level WUE<sub>i</sub> with EWUE, SWUE, IWUE or SWUE' across sites.

## RESULTS

### Inter-population genetic variation inferred from the provenance trial

We found low inter-population genetic variation for WUE<sub>i</sub> and radial growth in *P. sylvestris* as indicated by small and poorly estimated variance components of population effects, which were non-significant (Table 1). However, when the population effect was partitioned into a fixed effect accounting for differences between countries of origin and a remaining random population effect, significant differences in WUE<sub>i</sub> emerged between Spanish and German populations ( $p < 0.05$ ). In particular, German populations showed a 3.2%

higher  $WUE_i$  than Spanish populations (Fig. 2). On the other hand, there were no significant differences in TRW between countries (Fig. 2), but Spanish populations showed a significantly lower tree height than German ones ( $9.12 \pm 0.27$  m vs.  $10.84 \pm 0.28$  m respectively; mean  $\pm$  SE) and experienced lower mortality ( $11.2 \pm 0.05\%$  vs.  $19.2 \pm 0.12\%$  respectively).

### **Spatial phenotypic variation inferred from natural stands**

The spatial phenotypic variation for  $WUE_i$  was highly significant and *ca.* ten-fold higher than the magnitude of inter-population genetic variation estimated at the provenance trial (Table 1).  $WUE_i$  varied across sites between  $85.26 \mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$  (Vallfogona) and  $113.01 \mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$  (Estamariu II), which translated into a relative maximum  $WUE_i$  difference of 33% among stands. Mean TRW varied between 0.51 mm (Valls de Valira II) and 2.51 mm (Gombreny), which translated into a relative maximum TRW difference of *ca.* 400% among stands. The spatial component of phenotypic variation for TRW was highly significant and about five-fold higher than that of inter-population genetic variation (Table 1).

$WUE_i$  did not correlate with any physiographic, climatic or edaphic characteristic across stands (Online Resource 4). The only exception was soil depth, which was negatively associated with  $WUE_i$  (Fig. 3a).  $WUE_i$  did not correlate with TRW across stands (Fig. 3b). On the other hand, TRW was negatively correlated with the ratio of summer to total precipitation, with annual potential evapotranspiration and with stand density (Online Resource 4). At tree level, we did not find significant relationships between  $WUE_i$  and age or height, but TRW was negatively associated with tree age (Online Resource 4).

### **Temporal phenotypic variation inferred from tree-ring chronologies**

$WUE_i$  increased significantly over the last 30 years, with similar rates in Arcalís and Seira (Online Resource 5). Particularly,  $WUE_i$  was 17% (Arcalís) and 11% (Seira) higher in

the period of 2004-2008 than at the beginning of the 1980s (1980-1984 period). Due to the positive effect of increased atmospheric CO<sub>2</sub> concentration on WUE<sub>i</sub> over time, the temporal (year) variance calculated through annually-resolved C<sub>a</sub> values was much higher than the inter-population genetic variance (over ten-fold higher) and slightly higher than the spatial phenotypic variance (Online Resource 6). Instead, the high-frequency signal of time-varying WUE<sub>i</sub> was estimated using an average C<sub>a</sub> value over the study period (1975-2008 in Arcalís and 1980-2009 in Seira). After recalculation, the increasing trend disappeared in Seira (Online Resource 5). The resulting C<sub>a</sub>-constant mean WUE<sub>i</sub> was 77.79 μmol CO<sub>2</sub> mol<sup>-1</sup> H<sub>2</sub>O in Seira and 85.00 μmol CO<sub>2</sub> mol<sup>-1</sup> H<sub>2</sub>O in Arcalís, and the temporal phenotypic variance became four-fold lower (9.57 vs. 40.77 (μmol CO<sub>2</sub> mol<sup>-1</sup> H<sub>2</sub>O)<sup>2</sup>) and only marginally significant ( $p=0.11$ ). Also, it was about four-fold lower than the magnitude of spatial variation, but still three-fold higher than the magnitude of inter-population genetic variation (Table 1). The variance component of year by site (spatiotemporal) interaction was not significant and had about the same magnitude of the variance component of population (genetic) effects (Table 1).

TRW showed a significant increase over time in Seira, but a significant decrease in Arcalís (Online Resource 5). The common temporal variance estimated across sites on non-detrended (absolute) TRW records was negligible, but the magnitude of year by site interaction was very relevant (higher than those of inter-population genetic variation and of spatial phenotypic variation) (Online Resource 6). After high-pass filtering, the common temporal variance for TRW<sub>res</sub> became sizeable ( $0.04 \pm 0.02$  mm<sup>2</sup>) and similar to the variance associated with year by site interaction (Table 1). In this regard, the temporal component of phenotypic variation for TRW<sub>res</sub> was also similar to that of inter-population genetic variation (Table 1).

The relationships between  $C_a$ -constant  $WUE_i$  or  $TRW_i$  and monthly climatic variables are summarized in Fig. 4. In Seira,  $C_a$ -constant  $WUE_i$  correlated positively with March precipitation and negatively with summer (June and August) precipitation and SPEI drought index. At this site,  $TRW_i$  correlated negatively with October temperature and positively with November temperature and August precipitation and SPEI. In Arcalís,  $C_a$ -constant  $WUE_i$  correlated negatively with spring to early-summer precipitation and SPEI (April and June) and positively with July temperature. It also showed a strong negative correlation with winter (previous December and current January) precipitation, SPEI and temperature.  $TRW_i$  correlated positively with January SPEI and April temperature, and with precipitation and SPEI in May-June.

#### **Ecosystem-level WUE**

The average ecosystem WUE (EWUE) estimated for the period of 2000-2014 varied between  $2.17 \text{ g C mm}^{-1} \text{ H}_2\text{O}$  (Seira) and  $3.66 \text{ g C mm}^{-1} \text{ H}_2\text{O}$  (Sant Llorenç) across sites. Alternatively, the average soil WUE (SWUE) varied between  $12.63 \text{ g C kg}^{-1} \text{ H}_2\text{O}$  (Espot) and  $19.68 \text{ g C kg}^{-1} \text{ H}_2\text{O}$  (St. Joan Abadesses) across sites. Neither EWUE nor SWUE correlated with leaf-level  $WUE_i$  (Fig. 5a,b). IWUE (i.e. EWUE corrected by atmospheric water demand) varied between  $11.02 \text{ g C hPa mm}^{-1} \text{ H}_2\text{O}$  (Seira) and  $23.00 \text{ g C hPa mm}^{-1} \text{ H}_2\text{O}$  (Sant Llorenç). IWUE did not correlate with leaf-level  $WUE_i$  (Fig. 5c). The modified SWUE values taking into account soil depth at stand level, that is, accounting for the total amount of water potentially available along the soil profile, varied between  $19.44 \text{ g C kg}^{-1} \text{ H}_2\text{O}$  (Espot) and  $76.48 \text{ g C kg}^{-1} \text{ H}_2\text{O}$  (St. Joan Abadesses). In this case, a significant positive correlation was found between SWUE' and  $WUE_i$  (Fig. 5d).

## DISCUSSION

### The extent of genetic and phenotypic variation of WUE<sub>i</sub> in *Pinus sylvestris*

Phenotypic diversity in functional traits, including intra-specific genetic variation and phenotypic plasticity, is called upon to play a central role in assisting forest populations to withstand future environmental conditions (Nicotra et al. 2010; Alberto et al. 2013; Bussotti et al. 2015). Our results demonstrate very limited genetic divergence in WUE<sub>i</sub> among *P. sylvestris* populations of the western part of the species distribution, in line with previous studies reporting lack of genetic differences in  $\Delta^{13}\text{C}$  among populations of this species (Palmroth et al. 1999; Brendel et al. 2002). *P. sylvestris* is not subjected to pervasive droughts and, thus, selective pressure towards a strong stomatal regulation of water losses may be irrelevant to shape physiological adaptations among populations for this species (Matías et al. 2017). In comparison, other European pines thriving in Mediterranean regions and exposed to substantial water shortage, such as *Pinus pinaster* or *P. halepensis*, are known to bear a large intra-specific genetic variation in WUE<sub>i</sub> (Voltas et al. 2008; Aranda et al. 2010). Specifically, Voltas et al. (2008) reported differences of *ca.* 26  $\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$  among populations of the drought-avoidant *P. halepensis*, which is almost three-fold higher than the range we estimated for *P. sylvestris* (*ca.* 10  $\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$ ). However, our results are not conclusive of lack of intra-specific genetic variation in WUE<sub>i</sub> in *P. sylvestris*. Indeed, they do not allow for an assessment of intra-population genetic variation in relation to that present among populations. Such a comparison would require testing the performance of different progenies within each population in a provenance/progeny trial, which is currently unavailable at the south-western distribution range of *P. sylvestris*.

On the other hand, we could identify a significant, albeit low, genetic differentiation in WUE<sub>i</sub> after grouping populations according to their country of origin, which might suggest a role of selection on functional traits that contribute to WUE<sub>i</sub> (i.e. assimilation rate and stomata

control), but only at large (i.e. continental) scales. Particularly, German populations showed increased  $WUE_i$  coupled with a greater height growth compared with Spanish populations. This outcome agrees with an evidence for genetic differentiation in  $WUE$  reported in *P. sylvestris* for a subset of European populations, although at the seedling stage (Bachofen et al. 2018). These results may indicate improved carbon uptake through photosynthesis as mechanism enhancing  $WUE_i$  at the genetic level (and hence, primary growth) for this species (Fardusi et al. 2016).

We estimated, for a small Scots pine area (~5,000 km<sup>2</sup>), a phenotypic variation of  $WUE_i$  over ten-fold larger than the extent of variation related to inter-population genetic differentiation for two major European regions occupying together over 800,000 km<sup>2</sup>. Phenotypic variation in natural stands is the result of the interacting effects of genetic variation and phenotypic plasticity, but our approach could not directly disentangle the relative importance of these components. Despite this caveat, results from the provenance trial suggests lack of relevant population differentiation in  $WUE_i$  continent-wide for *P. sylvestris*, making unlikely the presence of a significant inter-population differentiation at the much smaller scale of the sampled natural stands. In this regard, two populations evaluated in the provenance trial, Morrano and Pobla de Lillet (Online Resource 1), which originated from the area where the 30 natural stands were sampled, did not differ in terms of  $WUE_i$  ( $89.09 \pm 2.46 \mu\text{mol mol}^{-1}$  vs.  $91.14 \pm 2.62 \mu\text{mol mol}^{-1}$ ), TRW ( $3.32 \pm 0.26$  mm vs.  $3.47 \pm 0.26$  mm) and height ( $8.67 \pm 0.74$  m vs.  $8.41 \pm 0.74$  m). This observation supports a lack of genetic differentiation among stands for the study traits. Although it can be argued that our results do not quantify within-population genetic variation in  $WUE_i$ , the sampling strategy adopted in the natural stands, consisting of a number of representative trees sampled per site, buffered possible genetic differences among trees. Therefore, the reported phenotypic variation among natural stands is most probably indicative of plastic responses to divergent growing conditions. The

large phenotypic plasticity for *P. sylvestris* is somewhat expected since pines are known to be extremely plastic organisms (e.g. Tapias et al. 2004), and suggests that Scots pine performance is primarily modulated by plastic responses in functional characteristics such as WUE<sub>i</sub> (Feichtinger et al. 2017), rather than by genetic adaptation. Other drought-related traits such as leaf-to-sapwood area ratio (Martínez-Vilalta et al. 2009) or wood anatomy (Eilmann et al. 2009; Martín et al. 2010) also show high plasticity in *P. sylvestris*.

Notably, climate characteristics did not correlate with WUE<sub>i</sub> across stands, despite the sampled sites differed considerably in terms of total precipitation, evapotranspiration and summer drought intensity. As a comparison, Ferrio and Voltas (2005) found that WUE<sub>i</sub> negatively correlated with annual precipitation across an aridity gradient of the xeric *P. halepensis* in eastern Iberian Peninsula. Coupling our data with those from Ferrio and Voltas (2005), a decay pattern of plastic WUE<sub>i</sub> responses to annual precipitation is evident across species (Fig. 6). The environmental conditions in which these two isohydric pines overlap in the Iberian Peninsula (~800 mm of annual precipitation) roughly agree with the threshold at which WUE<sub>i</sub> (and stomatal sensitivity) becomes unresponsive to precipitation (Fig. 6) or water deficit (Fig. 6 insert), as observed for *P. halepensis* (del Castillo et al. 2015). Within such a continuous distribution of pines, precipitation would mainly drive phenotypic variation of WUE<sub>i</sub> in *P. halepensis*, while *P. sylvestris* would be found in conditions where precipitation is no longer a fundamental determinant of spatial variation in gas exchange.

Instead, the driving force of phenotypic variation in WUE<sub>i</sub> for *P. sylvestris* was the amount of water available to roots, as indicated by the negative relationship between soil depth and WUE<sub>i</sub>. Because of methodological difficulties, the study of rooting patterns for elucidating adaptations to edaphic conditions is a much less explored field of research compared with the analysis of above-ground tree characteristics. However, the distribution of water along the soil profile is known to be extremely relevant in modulating plastic responses

of forest trees to drought (Lebourgeois et al. 2010; Song et al. 2015; Voltas et al. 2015). Song et al. (2015) found a lower increase of  $WUE_i$  under drought stress in old plantations of *P. sylvestris* compared to young stands, and explained the difference as a higher rooting depth of older trees and, therefore, an easier access to deep water sources. In our case, the depth of rooting zone, which can be regarded as proxy of water holding capacity, seems a critical characteristic for *P. sylvestris* growing at its south-western distribution edge (Mellert et al. 2017). However, other soil factors such as nutrient availability and its interactions with climate may also determine changes in  $WUE_i$  across sites, hence warranting further examination (Silva et al. 2015).

$WUE_i$  increased over the last 30 years by 11% and 17% at the mesic (Seira) and xeric site (Arcalís) respectively. These rates are consistent with previous results reporting similar  $WUE_i$  increments in European forests (Saurer et al. 2014) and in other *P. sylvestris* stands from the Iberian Peninsula (Andreu-Hayles et al. 2011; Voltas et al. 2013). After reappraising  $WUE_i$  for constant  $C_a$ , we observed increases over time only at Arcalís, indicating that warming-induced drought effects may effectively contribute to long-term  $WUE_i$  changes in drought-prone areas for this species. In fact, most sampling sites of the central and eastern Pyrenees showed higher mean  $WUE_i$  than Arcalís over the same period ( $93 \mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$ ) (cf. Fig. 3a), which suggests that water availability is the most limiting factor for regional tree performance. It can therefore be hypothesised that a long-term temporal variation of  $WUE_i$  related to increasing drought effects (Shestakova et al. 2017) may be common to most *P. sylvestris* stands of the region.

The lack of interaction between spatial and temporal plastic responses in terms of  $WUE_i$  anticipates the existence of common temporal patterns across sampling sites. Although this point would need further assessment, the fact that Arcalís and Seira shared the dependence of  $WUE_i$  on summer precipitation indicates a predominant role of summer water



availability influencing inter-annual variation of  $WUE_i$ . However,  $WUE_i$  was also negatively correlated with winter precipitation at the xeric site (Arcalís), which can be interpreted as the effect of groundwater recharge on tree performance during the growing season (Shestakova et al. 2014). Indeed, water stress could be delayed or suppressed during the growing season in years with important snow coverage, resulting in low  $WUE_i$ . Thus, our results suggest that soil water plays an important role in modulating inter-annual variation of  $WUE_i$ , at least at the xeric site, which is supportive of a strong dependence of spatial variation of  $WUE_i$  on soil depth for *P. sylvestris*.

### **Inter-population genetic differentiation and phenotypic variation of radial growth**

The extent of phenotypic variation and the magnitude of inter-population genetic differentiation in  $WUE_i$  were compared with those of radial growth as indicator of overall performance in forest trees. Tree growth is indeed influenced by many biotic, abiotic and stand (local) factors, but climate plays a major role in driving growth variation in *P. sylvestris* (Matías and Jump 2012; Sánchez-Salguero et al. 2015). Broadly speaking, the phenotypic variation in radial growth is predominantly driven by drought at the southern edge of the species distribution (Camarero et al. 1998; Matías and Jump 2012) and by low temperatures in boreal latitudes (Grace and Norton 1990; Persson and Beuker 1997; Matías and Jump 2012). However, there is less clear-cut information quantifying the contribution of plastic and genetic responses to this variation. In our study, we estimated the amount of inter-population genetic variation in radial growth to be about one-fifth and one-half the spatial and temporal phenotypic variation respectively. As for  $WUE_i$ , this finding suggests preponderance of plastic variation (especially at spatial level) over population differentiation, but with smaller differences in the case of radial growth.

## Upscaling $WUE_i$ to the ecosystem level

To understand the relevance of leaf-level  $WUE_i$  in determining ecosystem-level  $WUE$ , we attempted at scaling up our long-term spatial  $WUE_i$  estimates based on tree rings (2000-2007 period) using remote sensing data. This could be undertaken owing to comparatively homogeneous stand conditions across sites. Indeed, the sampled forests were mature, even-aged and unmanaged, with large basal areas and crown covers exceeding 65%, hence suggesting comparable leaf area indices (Whitehead 1978).

We used high resolution ( $\sim 1 \text{ km}^2$ ) estimates of GPP and ET derived from MODIS satellite data, which are considered reliable at large (i.e. regional or continental) scales. The use of MODIS GPP and ET at local (i.e. site) scale is instead more problematic. However, reliable site-level estimates of MODIS GPP and ET have been reported in evergreen-needle forests similar to our stands (Turner et al. 2006a; Turner et al. 2006b; Kim et al. 2012; Cristiano et al. 2015). Anyhow, absolute values of productivity and evapotranspiration have relatively little importance in our study, which is addressed to assess variation in carbon-water balance among stands. In this regard, a bias in GPP or ET estimations, if present, would likely be consistent across sites due to homogenous vegetation type and stand structure.

Although estimates of ecosystem  $WUE$  ( $EWUE$ ) varied considerably among stands, they were similar to values reported for evergreen-needle forests derived from either flux tower measurements (Tang et al. 2014) or remote sensing data (He et al. 2017). It should be noted, however, that  $EWUE$  is not able to directly catch the influence of available soil water on ecosystem performance, which was instead the primary driver of variation in  $WUE_i$  along the study area. He et al. (2017) proposed a new indicator ( $SWUE$ , or ratio of gross productivity to soil water content) to potentially improve the information on how ecosystems use available water. In the present study,  $SWUE$  was calculated from remotely sensed data of soil water content ( $SWC$ ) previously validated for the north-eastern Iberian Peninsula (Merlin

et al. 2013). The derived SWUE values were much higher than those reported by He et al. (2017). The authors found an average SWUE of 3.4 g C kg<sup>-1</sup> H<sub>2</sub>O for evergreen-needle forests, while our values varied between 12.63 and 19.68 g C kg<sup>-1</sup> H<sub>2</sub>O. Such discordance may be related to the very large difference in data resolution (1 km<sup>2</sup> in this work compared with ~ 600 km<sup>2</sup> in He et al. [2017]), which may have influenced site-level SWUE estimation. This issue, however, would require specific attention that is beyond the scope of our study.

None of EWUE, SWUE or IWUE (i.e. inherent WUE) (Beer et al. 2009) were related to variation in WUE<sub>i</sub> among stands. On the other hand, when SWUE was corrected to account for soil depth, the resulting SWUE' significantly correlated with WUE<sub>i</sub> ( $p < 0.01$ ), indicating a tight relation between leaf-level physiology and gross productivity per unit of soil water available. This outcome suggests that the range of variation in leaf-level WUE<sub>i</sub>, as inferred from tree rings, can be upscaled to ecosystem level if accounting for soil water holding capacity *via* soil depth. Indeed, many studies have demonstrated the influence of soil water availability on canopy conductance, an effect that is remarkably similar across tree species (e.g. Granier et al. 2000). Our finding suggests a direct influence of plastic variation of WUE<sub>i</sub> of a major forest species in determining ecosystem water and carbon balances. On the other hand, local factors controlling WUE<sub>i</sub> (such as rooting depth) must be taken into account when scaling up from leaf to ecosystem WUE.

## CONCLUSIONS

Accurate predictions of water-use efficiency changes in forests are crucial to understand how climate change will affect terrestrial carbon and water balances. While the two components –genetic and plastic– of phenotypic variation of WUE<sub>i</sub> can potentially influence ecosystem WUE, our results point to a minor role of genetic differentiation in the variation of WUE<sub>i</sub> in *P. sylvestris*. Instead, a maximum 33%–difference in WUE<sub>i</sub> among

stands indicates a strong spatial divergence in the water and carbon economy of pinewoods in a small area of *ca.* 5000 km<sup>2</sup>. We also showed that long-term spatial differentiation in ecosystem WUE can be directly coupled to carbon uptake and stomatal responses for closed forest canopies in an isohydric species such as *P. sylvestris*. The key role of soil water holding capacity modulating WUE at both leaf and ecosystem levels, at least in *P. sylvestris*, emphasizes the necessity to incorporate belowground information when forecasting forest responses to climate (Ostle et al. 2009; van der Putten et al. 2013).

Although the importance of inter-population genetic variation of WUE<sub>i</sub> seems marginal for understanding *P. sylvestris* responses to environmental changes, this observation could not hold true for other widespread isohydric pines (e.g. *P. halepensis* or *Pinus pinaster*) in which strong genetic variation for this trait is acknowledged (Voltas et al. 2008; Aranda et al. 2010). Altogether, rigorous assessments of global change impacts on forests should consider potential additive effects of genetic variation and phenotypic plasticity, as well as their interactions, in modulating phenotypic variation in functional traits such as WUE<sub>i</sub> (Benito-Garzón et al. 2011; Klein et al. 2013).

## COMPLIANCE WITH ETHICAL STANDARDS

The authors declare no conflict of interest.

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## FIGURES CAPTIONS

**Fig. 1** (a) Location of the provenance trial (Aragüés del Puerto, Spanish Pyrenees) (black square) and geographic origin of the German (green squares) and Spanish (red squares) populations evaluated in the trial. The climograph of the trial site is also included (left insert). The right insert shows the study area in which the natural stands (blue circles) were sampled. Sites chosen to build temporal chronologies (Arcalís and Seira) are indicated with empty blue circles in the right insert. (b) Mean annual precipitation and temperature for the distribution range of *P. sylvestris* in Europe (grey circles) calculated in 10' resolution grids from the WorldClim database (period 1960-1990). The species range is derived from the EUFORGEN distribution map (<http://www.euforgen.org/species/pinus-sylvestris>). Temperature and precipitation of the trial site (black square), of German and Spanish sites of origin (red and green squares), of natural stands (blue circles) and of two chronologies sites (open blue circles) are shown

**Fig. 2** Mean intrinsic water-use efficiency ( $WUE_i$ ) and tree-ring width (TRW) of Spanish and German populations of the provenance trial. Error bars indicate standard errors. The asterisk indicates a statistically significant difference at  $p < 0.05$

**Fig. 3** Relationships between intrinsic water-use efficiency ( $WUE_i$ ) and soil depth (a) and between  $WUE_i$  and tree-ring width (TRW) (b) in the 30 natural stands composing the study area. Each dot indicates one particular stand. Open dots indicate the two sites where temporal chronologies were available. Error bars indicate standard errors

**Fig. 4** Bootstrapped correlations between intrinsic water-use efficiency ( $WUE_i$ , top panels) or indexed tree-ring width ( $TRW_i$ , bottom panels) and precipitation, temperature and SPEI drought index in Arcalís (1975-2008 period) and Seira (1980-2009 period). Correlations were calculated on a monthly basis from October of the previous year to September of the current year. Filled bars represent significant correlations

**Fig. 5** Relationships between  $WUE_i$  and ecosystem water-use efficiency (EWUE) (a), soil water-use efficiency (SWUE) (b), inherent water-use efficiency (IWUE) (c) and soil water-use efficiency corrected for soil depth (SWUE') (d) in the 30 natural stands composing the study area. Each dot indicates one particular stand. Open dots indicate the two sites where temporal chronologies were available

**Fig. 6** Regression plot (negative exponential function) of  $WUE_i$  on mean annual precipitation combining records of *Pinus sylvestris* (present study; closed circles) and *Pinus halepensis* stands (original data published in Ferrio and Voltas (2005); open circles) sampled across north-eastern Iberian Peninsula.  $WUE_i$  was estimated from carbon isotope discrimination ( $\Delta^{13}C$ ) of tree rings corresponding to different time periods (2000-2007 for *P. sylvestris*; 1975-1999 for *P. halepensis*).  $WUE_i$  values were estimated using the mean atmospheric  $CO_2$  concentration ( $C_a$ ) for the period of 1975-2007 in order to allow a direct comparison of both datasets. We assumed constant  $\Delta^{13}C$  values over time (i.e.  $C_i/C_a$  is maintained constant), as previously observed in the Iberian Peninsula for Scots pine (Voltas et al. 2013; Hereş et al. 2014). The insert shows the regression plot of  $WUE_i$  on the ratio of annual precipitation to annual potential evapotranspiration. Both regressions are significant at  $p < 0.01$  (\*\*)

## TABLES

**Table 1. Linear mixed-effects models for intrinsic water-use efficiency (WUE<sub>i</sub>) and tree-ring width (TRW).** Data were obtained from the provenance trial of Aragüés del Puerto, a set of 30 natural stands located in the central and eastern Pyrenees mountains and two tree-ring chronologies from the same area. For chronologies, variance components were estimated based on C<sub>a</sub>-constant WUE<sub>i</sub> values or high-pass filtered TRW residuals (TRW<sub>res</sub>). Only the random effects of the models are shown.

	Source of variation	Variance component	% of total variance	Chi-Square value <sup>a</sup>	Likelihood ratio test ( <i>p</i> -value) <sup>a</sup>
<b>WUE<sub>i</sub></b>					
Provenance trial					
	Population	3.38±3.73 <sup>b</sup>	11.20	0.87	<i>p</i> =0.35
	Population×Block	4.14±4.50	13.73	1.14	<i>p</i> =0.28
	Residual	22.68±3.87	75.07		
Natural stands					
	Site	34.47±12.37 <sup>c</sup>	50.77	38.63	<i>p</i> <0.01
	Residual	33.42±5.41	49.23		
Chronologies					
	Tree [Site]	20.53±8.40	40.65	52.45	<i>p</i> <0.01
	Year	9.57±5.27 <sup>d</sup>	18.94	2.45	<i>p</i> =0.11
	Year×Site	3.69±3.26 <sup>e</sup>	7.30	3.52	<i>p</i> =0.06
	Residual	16.72±2.34	33.11		
<b>TRW</b>					
Provenance trial					
	Population	0.03±0.02 <sup>b</sup>	3.97	3.70	<i>p</i> =0.05
	Population×Block	0.07±0.02	10.31	32.81	<i>p</i> <0.01
	Residual	0.58±0.03	85.72		
Natural stands					
	Site	0.15±0.05 <sup>c</sup>	54.27	31.67	<i>p</i> <0.01
	Residual	0.13±0.02	45.73		
Chronologies					
	Tree [Site]	0.00±0.00	0.00	0.00	<i>p</i> =1.00
	Year	0.04±0.02 <sup>d</sup>	10.11	7.64	<i>p</i> <0.01
	Year×Site	0.03±0.01 <sup>e</sup>	7.30	30.66	<i>p</i> <0.01
	Residual	0.32±0.01	82.59		

<sup>a</sup> Test for the null hypothesis of variance component being equal to 0; <sup>b</sup> estimate of inter-population genetic variation; <sup>c</sup> estimate of spatial phenotypic variation; <sup>d</sup> estimate of temporal phenotypic variation; <sup>e</sup> estimate of spatiotemporal phenotypic variation

1010	<b>Electronic Supplementary Material</b>
1	
2	1011 <b>Online Resource 1</b> Characteristics of the 22 Scots pine populations evaluated in the
3	
4	1012 provenance trial.
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7	1013 <b>Online Resource 2</b> Characteristics of the 30 Scots pine natural stands.
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9	1014 <b>Online Resource 3</b> Normal quantile plots of the ANOVAs residuals
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11	121015 <b>Online Resource 4</b> Pearson correlations between WUE <sub>i</sub> or TRW and climatic, edaphic and
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13	141016 physiographic characteristics of the 30 Scots pine natural stands.
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15	171017 <b>Online Resource 5</b> WUE <sub>i</sub> and TRW chronologies.
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17	191018 <b>Online Resource 6</b> Variance components estimated for absolute TRW and for WUE <sub>i</sub>
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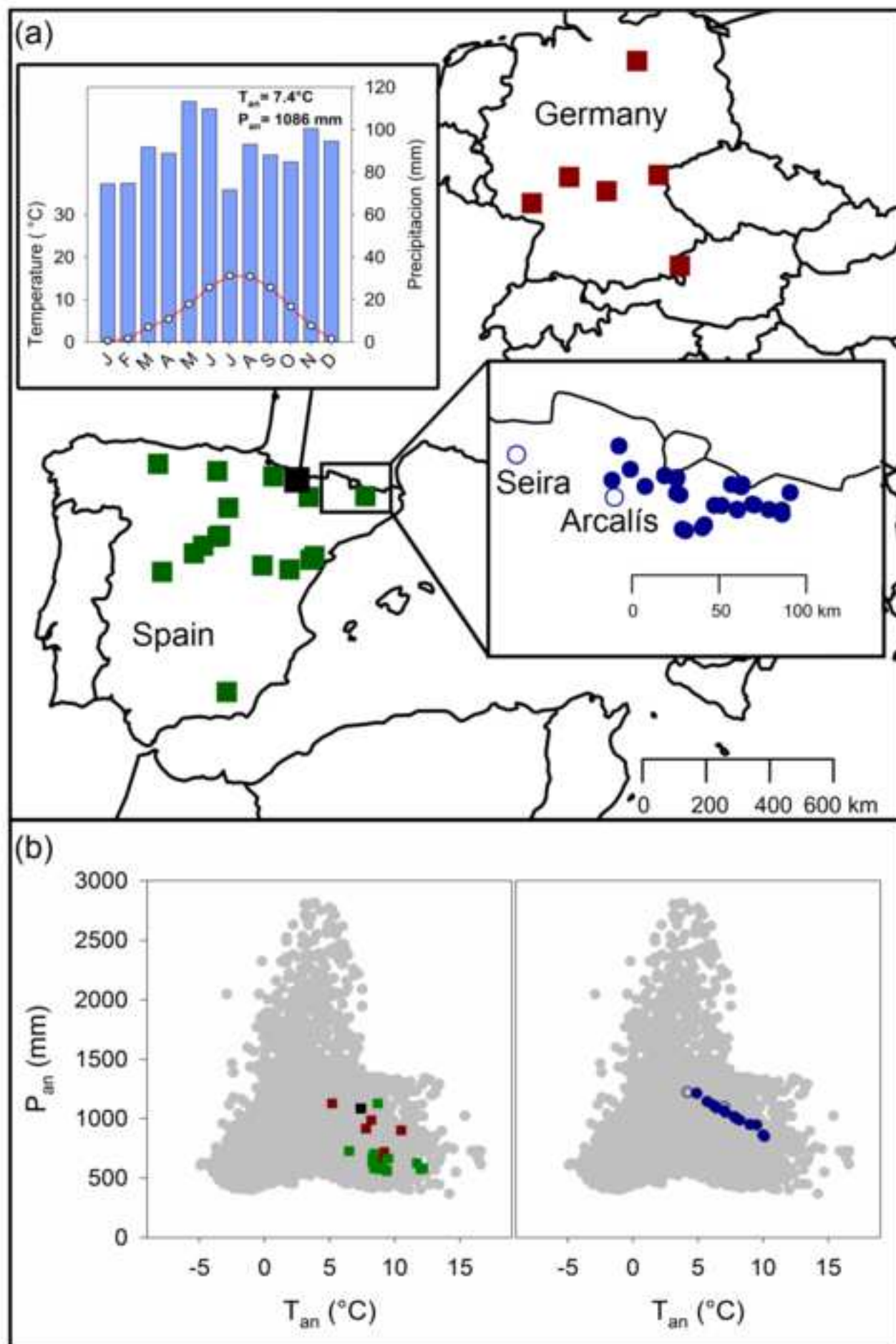


Figure 2

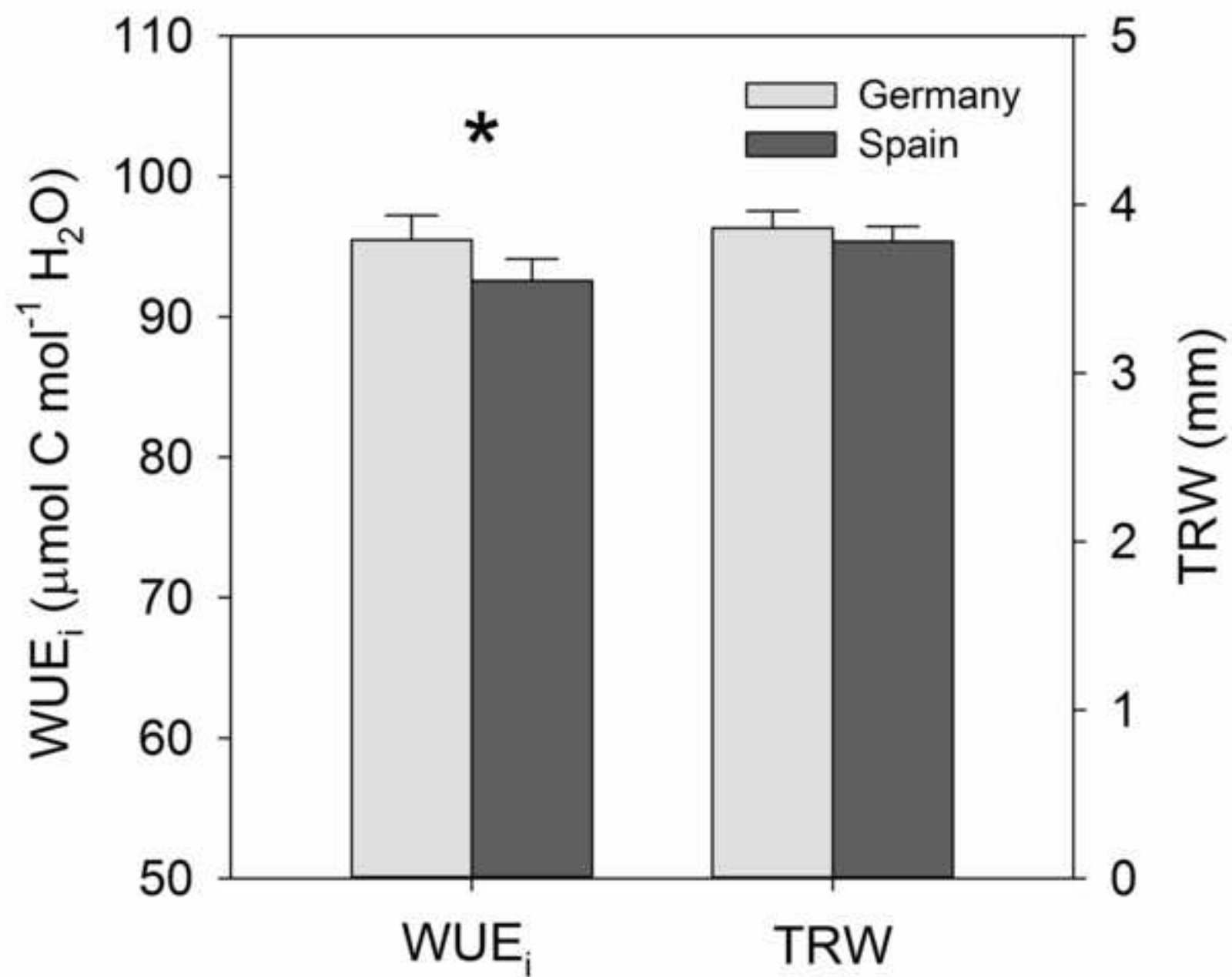


Figure 3

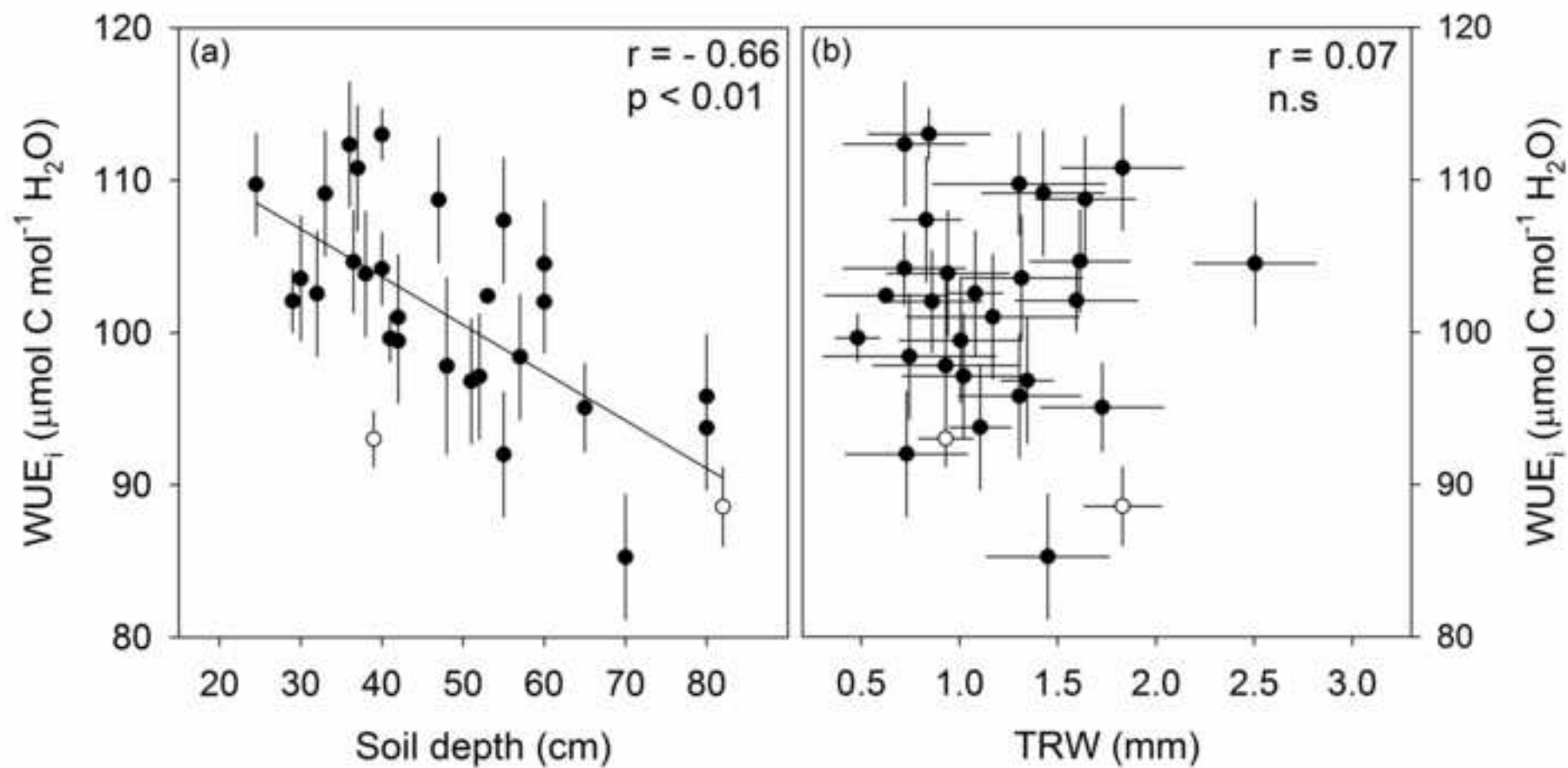




Figure 4

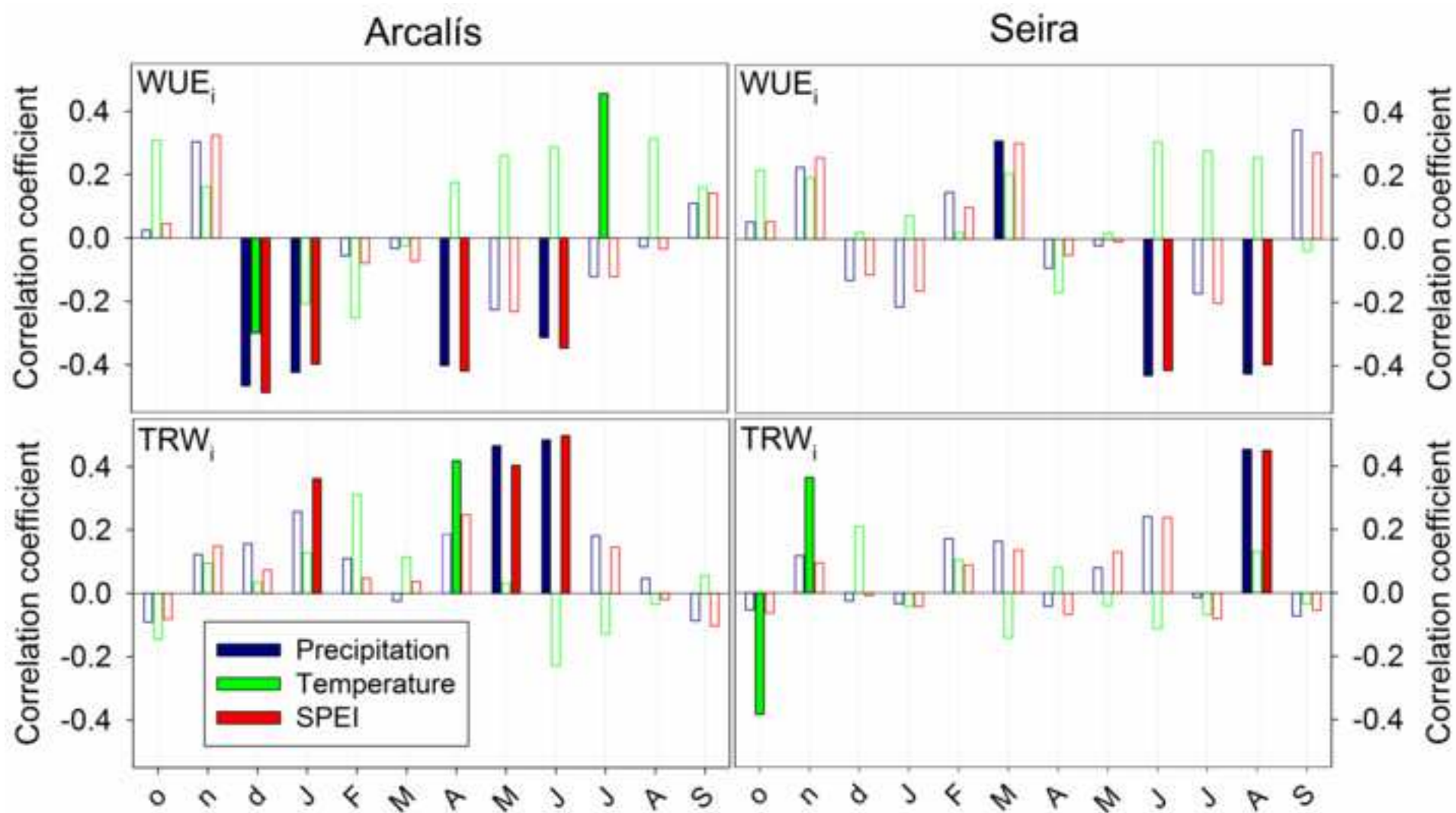


Figure 5

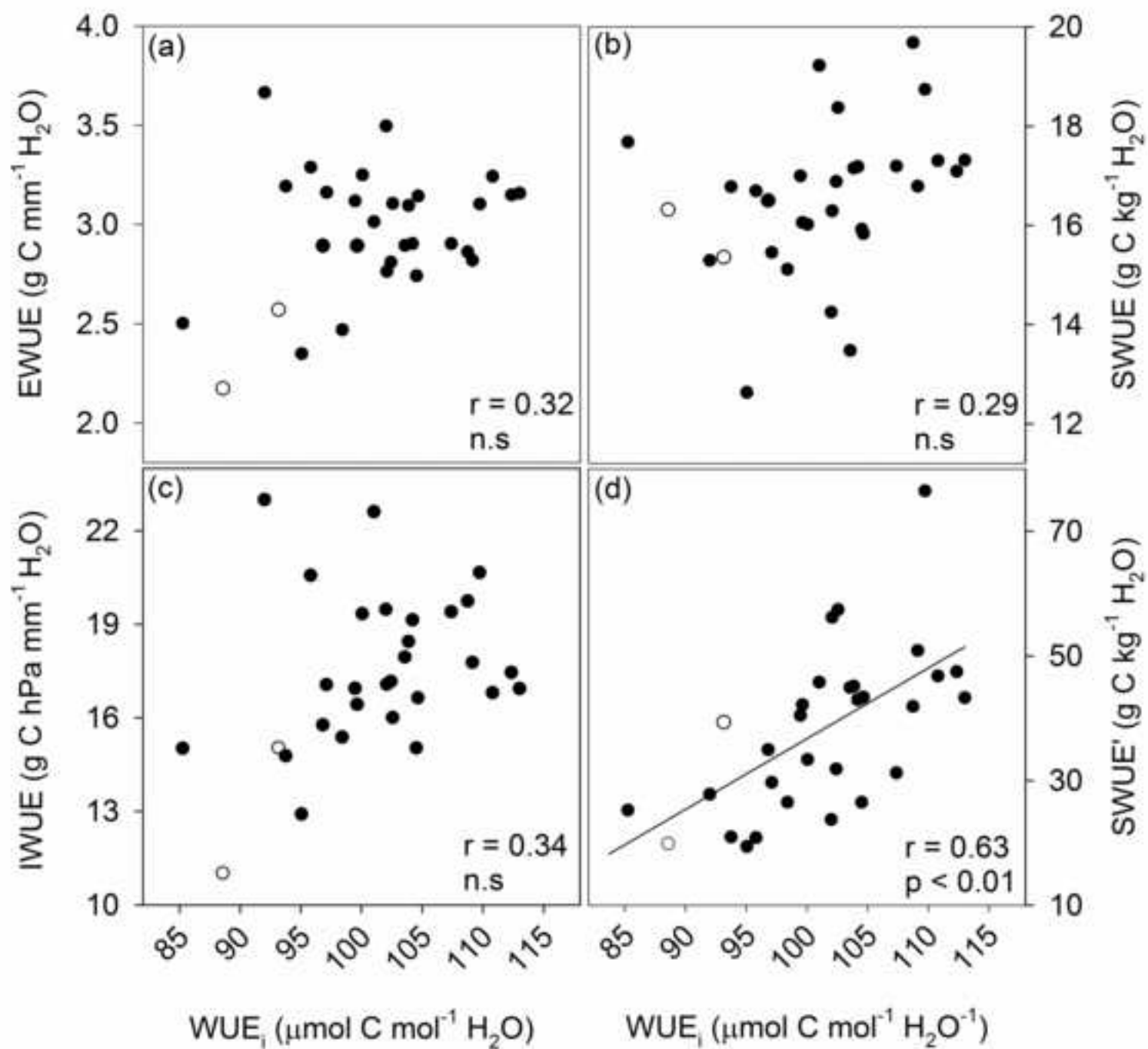
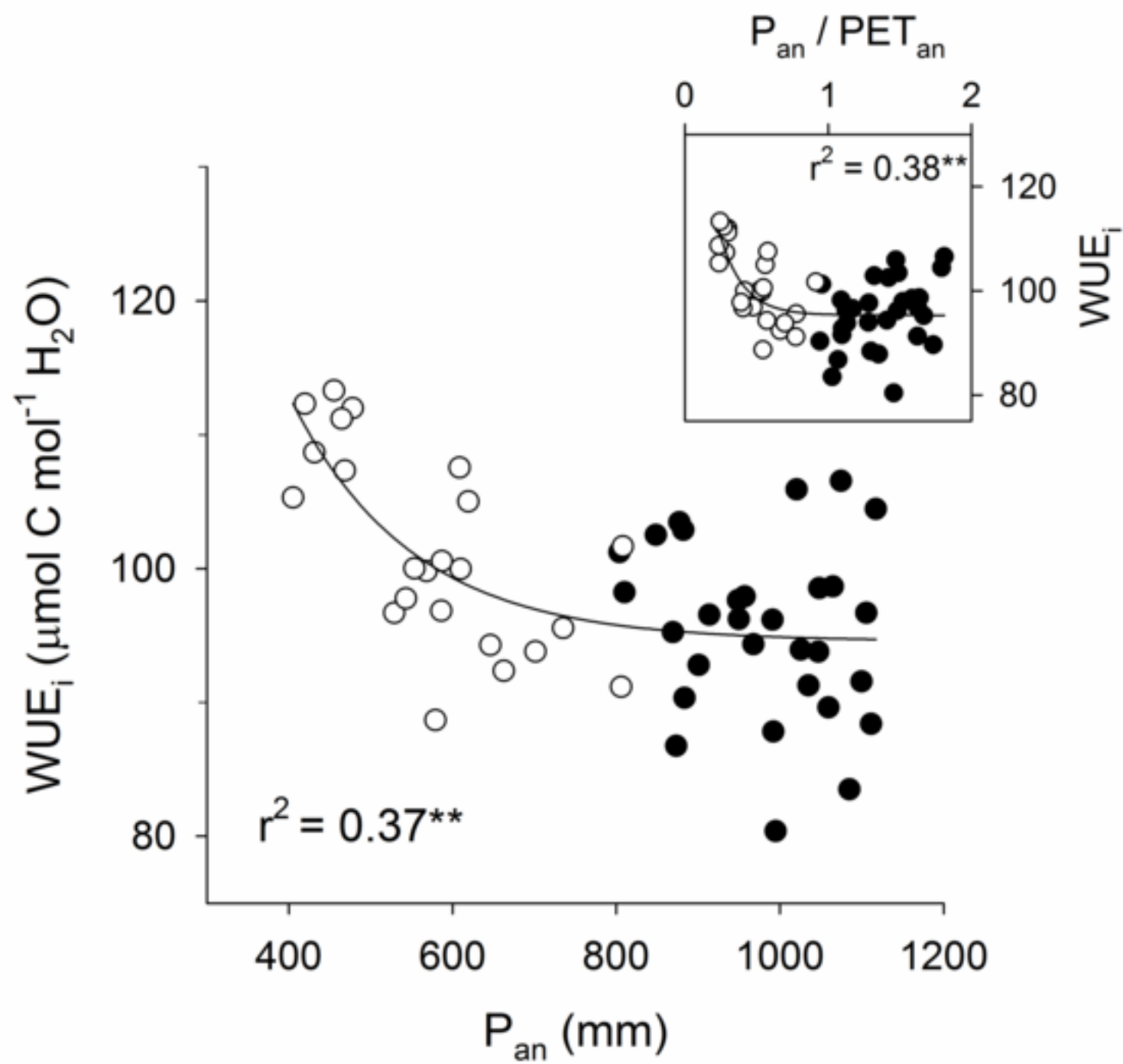


Figure 6



**Title** Scarce population genetic differentiation but substantial spatiotemporal phenotypic variation of water-use efficiency in *Pinus sylvestris* at its western distribution

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Main characteristics of the 22 populations of *Pinus sylvestris* evaluated in the provenance trial of Aragiüés del Puerto (Spanish Pyrenees). Data refer to the site of origin of each population.

Country	Provenance	Latitude	Longitude	Elevation (m a.s.l.)	T <sub>an</sub> (°C)	P <sub>an</sub> (mm)	P <sub>s</sub> (mm)	PET <sub>an</sub> (mm)
Germany	Gartow	53° 02' 03" N	11° 25' 03" E	50	8.7	582	186	698
Germany	Laufen	47° 57' 08" N	12° 54' 00" E	430	8.7	1113	423	839
Germany	Otterberg	49° 30' 03" N	07° 45' 02" E	300	8.5	681	205	764
Germany	Selb	50° 12' 03" N	12° 10' 03" E	570	6.5	702	235	683
Germany	Wiesentheid	49° 48' 03" N	10° 21' 03" E	220	8.8	611	203	793
Germany	Wolfgang	50° 09' 03" N	09° 03' 02" E	177	9.5	662	204	795
Spain	Baza	37° 22' 31" N	02° 51' 34" W	2050	8.1	783	96	1000
Spain	Borau	42° 41' 56" N	00° 34' 44" W	1550	5.9	1093	238	737
Spain	Campisábalos	41° 14' 54" N	03° 05' 34" W	1400	8.5	591	113	930
Spain	Castell de Cabres	40° 38' 57" N	00° 03' 19" E	1150	10.4	649	132	876
Spain	Covaleda	41° 56' 41" N	02° 48' 40" W	1550	7.6	748	147	889
Spain	Galve de Sorbe	41° 13' 14" N	03° 10' 45" W	1400	8.9	592	105	938
Spain	Gúdar	40° 24' 41" N	00° 41' 04" W	1700	7.3	710	163	815
Spain	La Sènia	40° 44' 50" N	00° 11' 29" E	1100	9.9	638	142	858
Spain	Morrano	42° 12' 42" N	00° 05' 54" W	700	8.9	593	178	851
Spain	Navafría	41° 00' 20" N	03° 47' 56" W	1600	10.6	679	78	976
Spain	Navarredonda	40° 21' 18" N	05° 06' 50" W	1550	8.6	603	84	950
Spain	Orihuela	40° 30' 47" N	01° 37' 46" W	1750	6.9	691	150	874
Spain	Pobla de Lillet	42° 13' 46" N	01° 57' 30" E	1100	9.7	895	245	788
Spain	Puebla de Lillo	43° 03' 35" N	05° 15' 12" W	1550	8	1005	161	850
Spain	San Zadornil	42° 51' 11" N	03° 11' 43" W	1000	9.8	935	162	837
Spain	Valsaín	40° 48' 43" N	04° 00' 33" W	1550	8.3	589	100	911

T<sub>an</sub> (mean annual temperature); P<sub>an</sub> (total annual precipitation); P<sub>s</sub> (June to August precipitation); PET<sub>an</sub> (annual potential evapotranspiration)

Title Scarce population genetic differentiation but substantial spatiotemporal phenotypic variation of water-use efficiency in *Pinus sylvestris* at its western

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Main characteristics of the 30 *Pinus sylvestris* natural stands composing the study area (central/eastern Pyrenees, NE Spain).

Site	Latitude	Longitude	Elevation (m a.s.l.)	T <sub>an</sub> (°C)	P <sub>an</sub> (mm)	P <sub>s</sub> (mm)	PET <sub>an</sub> (mm)	Basal area (m <sup>2</sup> ha <sup>-1</sup> )	Density (tree ha <sup>-1</sup> )	Crown cover (%)	Height (m)	DBH (cm)	Age (year)	Rocky outcrops (%)	Rock fragments in soil (%)
Alàs i Cercs (I)	42° 19' 26" N	01° 30' 32" E	1130	9.2	914	256	804	46.2	2500	75	12.5	15.3	50	0	15
Alàs i Cercs (II)	42° 18' 54" N	01° 31' 55" E	1391	7.6	1021	270	741	56.4	1850	80	11.8	19.7	58	0	20
Alp (I)	42° 22' 08" N	01° 53' 24" E	1356	8.9	949	256	773	42.7	1500	80	15.2	19	48	0	40
Alp (II)	42° 21' 25" N	01° 57' 32" E	1468	7	1065	267	705	29.7	725	72	16.6	22.8	64	30	20
Arcalís	42° 22' 24" N	01° 11' 00" E	1150	7.1	1058	276	810	10	1070	50	10.9	26.5	80	20	20
Castellar de n'Hug	42° 16' 01" N	02° 02' 06" E	1314	8.7	968	252	755	37.5	1100	70	12.3	20.8	76	0	5
Codó	42° 08' 17" N	01° 32' 56" E	1300	7.9	991	258	747	44.2	1760	80	20.2	37.5	62	0	35
Espot	42° 34' 05" N	01° 06' 36" E	1324	7.6	1059	282	764	51.7	1219	75	17.7	23.4	54	5	20
Estamariu (I)	42° 24' 18" N	01° 30' 43" E	1609	6.3	1106	283	697	45.7	950	32	15.6	24.7	96	0	60
Estamariu (II)	42° 23' 53" N	01° 30' 36" E	1558	6.8	1075	279	716	46.8	2100	80	12.1	16.8	51	0	50
Fontanals (I)	42° 21' 43" N	01° 56' 42" E	1702	6.2	1117	274	678	43.1	950	65	14.7	24	56	10	20
Fontanals (II)	42° 22' 12" N	01° 57' 43" E	1265	6.3	1111	272	681	43.1	650	70	17.7	29.1	56	5	40
Gisclareny (I)	42° 15' 40" N	01° 46' 19" E	1405	8.7	950	256	767	50.3	1500	80	15.8	20.7	50	5	30
Gisclareny (II)	42° 15' 36" N	01° 49' 16" E	1082	9.8	882	247	803	36.3	1600	70	14	17	67	0	40
Gombreny	42° 15' 47" N	02° 02' 53" E	1242	8.9	957	250	761	41.2	1250	80	15.5	20.5	48	0	20
Guils	42° 21' 32" N	01° 17' 28" E	1463	7.2	1048	276	740	58	1050	70	18.7	26.5	76	5	65
Guixers (I)	42° 09' 36" N	01° 42' 11" E	1523	7.1	1047	264	712	22.8	550	70	16	23	67	0	50
Guixers (II)	42° 08' 42" N	01° 41' 17" E	1112	9.6	884	244	801	45	1100	75	16.6	22.8	70	0	5
Pobla de Lillet (I)	42° 14' 24" N	01° 55' 44" E	818	11.2	804	235	843	33.6	800	65	19.8	23.1	84	5	10
Pobla de Lillet (II)	42° 14' 13" N	01° 55' 55" E	803	11.1	810	236	840	52.1	750	65	24.4	29.7	74	5	35
Queralbs	42° 14' 20" N	02° 98' 53" E	1552	10.6	869	240	810	70.4	1550	75	15.2	24.1	51	5	35
Rialb	42° 26' 49" N	01° 11' 17" E	1671	6.5	1100	285	717	51.9	1750	80	12.7	19.4	58	15	15
Sant Llorenç	42° 07' 48" N	01° 34' 12" E	1075	9.7	873	242	810	25.2	700	75	15.2	21.4	69	35	35
Seira	42° 31' 24" N	0° 23' 01" N	1538	6.1	1085	267	810	76.2	1025	75	17.7	37.38	66	20	10
Soriguera	42° 21' 00" N	01° 05' 20" E	954	9.7	901	252	854	35.8	750	70	15.1	24.7	61	0	40
St Joan Abadesses (I)	42° 12' 58" N	02° 14' 24" E	921	11.4	849	235	830	30.3	500	70	17.7	27.8	94	20	0
St Joan Abadesses (II)	42° 14' 10" N	02° 14' 20" E	999	10.8	877	239	813	22.7	1162	65	10.3	15.9	72	0	10
Vallfogona	42° 19' 37" N	02° 17' 46" E	1407	8.6	995	247	744	35.5	700	75	18.2	25.4	49	0	0
Valls de Valira (I)	42° 24' 50" N	01° 25' 34" E	1357	7.5	1035	275	744	33.5	700	70	15	24.7	101	0	35
Valls de Valira (II)	42° 24' 58" N	01° 25' 34" E	1451	7.6	1026	274	751	36	2500	70	14.3	13.5	125	0	45

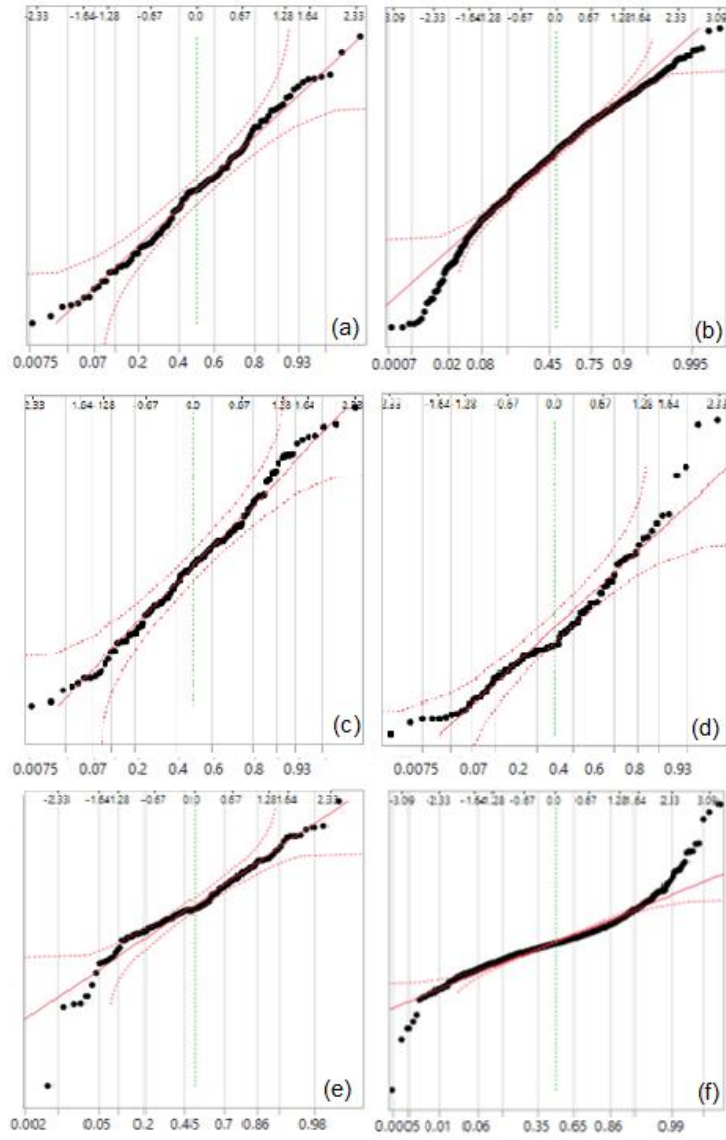
T<sub>an</sub> (mean annual temperature); P<sub>an</sub> (total annual precipitation); P<sub>s</sub> (June to August precipitation); PET<sub>an</sub> (annual potential evapotranspiration)

**European Journal of Forest Research**

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**Figure S1.** Normal quantile plots of the residuals of the ANOVAs fitted on WUE<sub>i</sub> (a) and TRW (b) in the provenance trial, WUE<sub>i</sub> (c) and TRW (d) in the natural sampled sites and WUE<sub>i</sub> (e) and TRW (f) in the temporal chronologies

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Pearson correlations between either intrinsic water-use efficiency (WUE<sub>i</sub>) or tree-ring width (TRW) and climatic, edaphic and physiographic site characteristics for 30 natural stands of *P. sylvestris* . Significant correlations ( $p < 0.05$ ) are highlighted in bold.

Variable	<i>r</i> (WUE <sub>i</sub> )	<i>p</i> -value	<i>r</i> (TRW)	<i>p</i> -value
Elevation	-0.08	0.66	-0.29	0.12
T <sub>an</sub>	0.22	0.23	0.19	0.33
T <sub>max</sub>	0.19	0.31	0.21	0.26
T <sub>min</sub>	0.25	0.18	0.16	0.41
P <sub>an</sub>	-0.19	0.3	-0.25	0.18
P <sub>s</sub>	-0.1	0.61	-0.05	0.79
P <sub>j</sub> /P <sub>an</sub>	0.28	0.13	<b>-0.55</b>	<b>&lt;0.01</b>
PET <sub>an</sub>	0.06	0.77	<b>-0.4</b>	<b>0.03</b>
Soil depth	<b>-0.66</b>	<b>&lt;0.01</b>	0.05	0.79
Basal area	0.2	0.31	-0.02	0.93
Density	0.28	0.15	<b>-0.43</b>	<b>0.02</b>
Slope	-0.09	0.64	-0.23	0.24
Position	-0.03	0.87	-0.13	0.51
Aspect	-0.1	0.61	-0.09	0.67
Soil carbonates	0.22	0.27	-0.1	0.62
Height <sup>a</sup>	-0.18	0.33	0.15	0.42
Age <sup>a</sup>	-0.15	0.12	<b>-0.39</b>	<b>&lt; 0.01</b>
TRW <sup>a</sup>	-0.04	0.66		

T<sub>an</sub> (mean annual temperature); T<sub>max</sub> (average maximum annual temperature); T<sub>min</sub> (average minimum annual temperature); P<sub>an</sub> (total annual precipitation); P<sub>j</sub> (June to August precipitation); PET<sub>an</sub> (annual potential evapotranspiration)

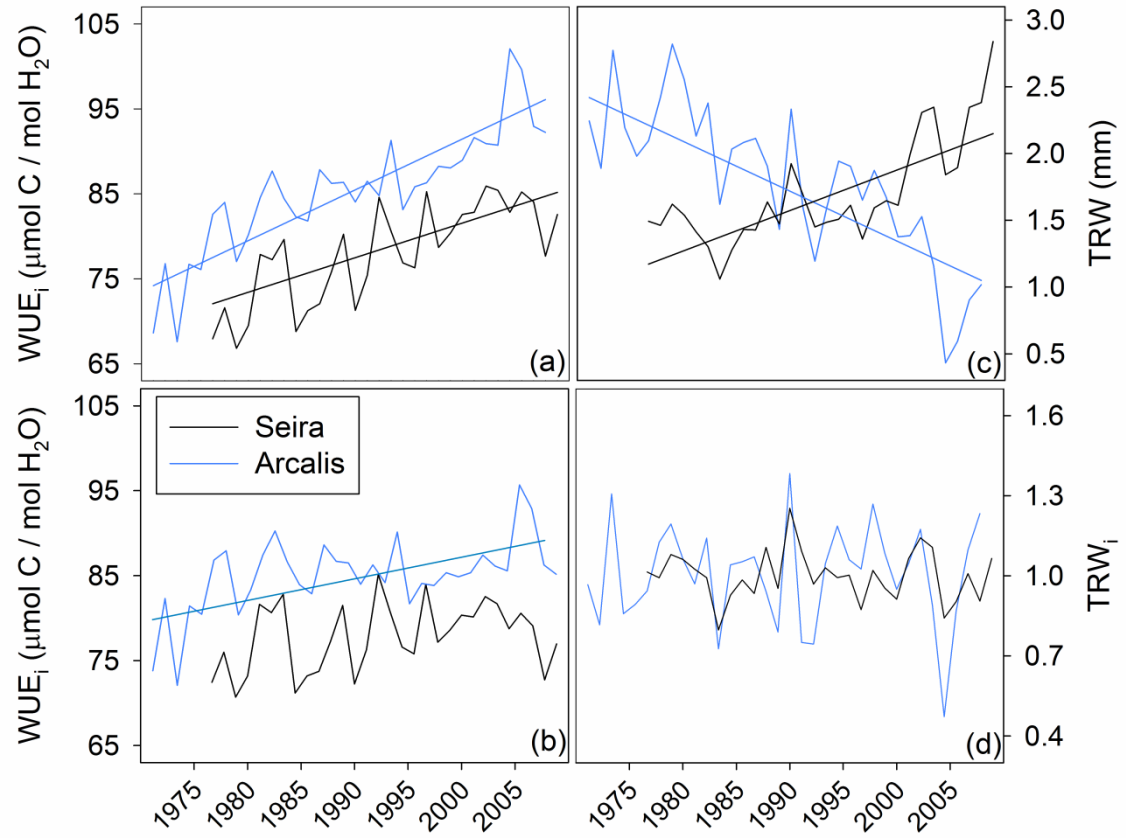


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**Figure S2.** Intrinsic water-use efficiency ( $WUE_i$ ) and tree-ring width ( $TRW$ ) chronologies in Seira and Arcalís. Left panels show  $WUE_i$  time series estimated using either annually-resolved  $C_a$  values (a) or the average  $C_a$  over the study period (b). Right panels show absolute  $TRW$  records (i.e. not high-pass filtered) (c) and  $TRW$  indices ( $TRW_i$ ) after high-pass filtering (d). Significant linear trends are depicted with lines ( $p < 0.05$ ).

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Linear mixed-effects models for intrinsic water-use efficiency (WUEi) and tree-ring width (TRW) for the provenance trial of Aragüés del Puerto, a set of 30 natural stands located in the central and eastern Pyrenees mountains and two temporal chronologies from the same area. For chronologies, variance components were estimated based on annually-resolved Ca WUEi values or absolute TRW records (not high-pass filtered) Only the random effects of the models are shown.

Source of variation		Variance component	% of total variance	Chi-Square value <sup>a</sup>	Likelihood ratio test ( <i>p</i> -value) <sup>a</sup>
<b>WUE<sub>i</sub></b>					
Provenance trial					
	Population	3.38±3.73 <sup>b</sup>	11.2	0.87	<i>p</i> =0.35
	Population×Block	4.14±4.50	13.73	1.14	<i>p</i> =0.28
	Residual	22.68±3.87	75.07		
Natural stands					
	Site	34.47±12.37 <sup>c</sup>	50.77	38.63	<i>p</i> <0.01
	Residual	33.42±5.41	49.23		
Chronologies <sup>b</sup>					
	Tree [Site]	20.89±8.58d	25.56	102.56	<i>p</i> <0.01
	Year	40.77±12.57e	49.88	19.96	<i>p</i> <0.01
	Year×Site	3.00±2.47	3.67	3.25	<i>p</i> =0.07
	Residual	17.07±2.51	20.89		
<b>TRW</b>					
Provenance trial					
	Population	0.03±0.02 <sup>b</sup>	3.97	3.7	<i>p</i> =0.05
	Population×Block	0.07±0.02	10.31	32.81	<i>p</i> <0.01
	Residual	0.58±0.03	85.72		
Natural stands					
	Site	0.15±0.05 <sup>c</sup>	54.27	31.67	<i>p</i> <0.01
	Residual	0.13±0.02	45.73		
Chronologies					
	Tree [Site]	0.55±0.10 <sup>d</sup>	40.64	1128.67	<i>p</i> <0.01
	Year	0.00±0.00 <sup>e</sup>	0	0	<i>p</i> =1.00
	Year×Site	0.25±0.05	18.45	346.96	<i>p</i> <0.01
	Residual	0.55±0.02	40.91		

<sup>a</sup> Test for the null hypothesis of variance component being equal to 0; <sup>b</sup> estimate of inter-population genetic variation; <sup>c</sup> estimate of spatial phenotypic variation; <sup>d</sup> estimate of temporal phenotypic variation; <sup>e</sup> estimate of spatiotemporal phenotypic variation